Supplementary Figures and Table

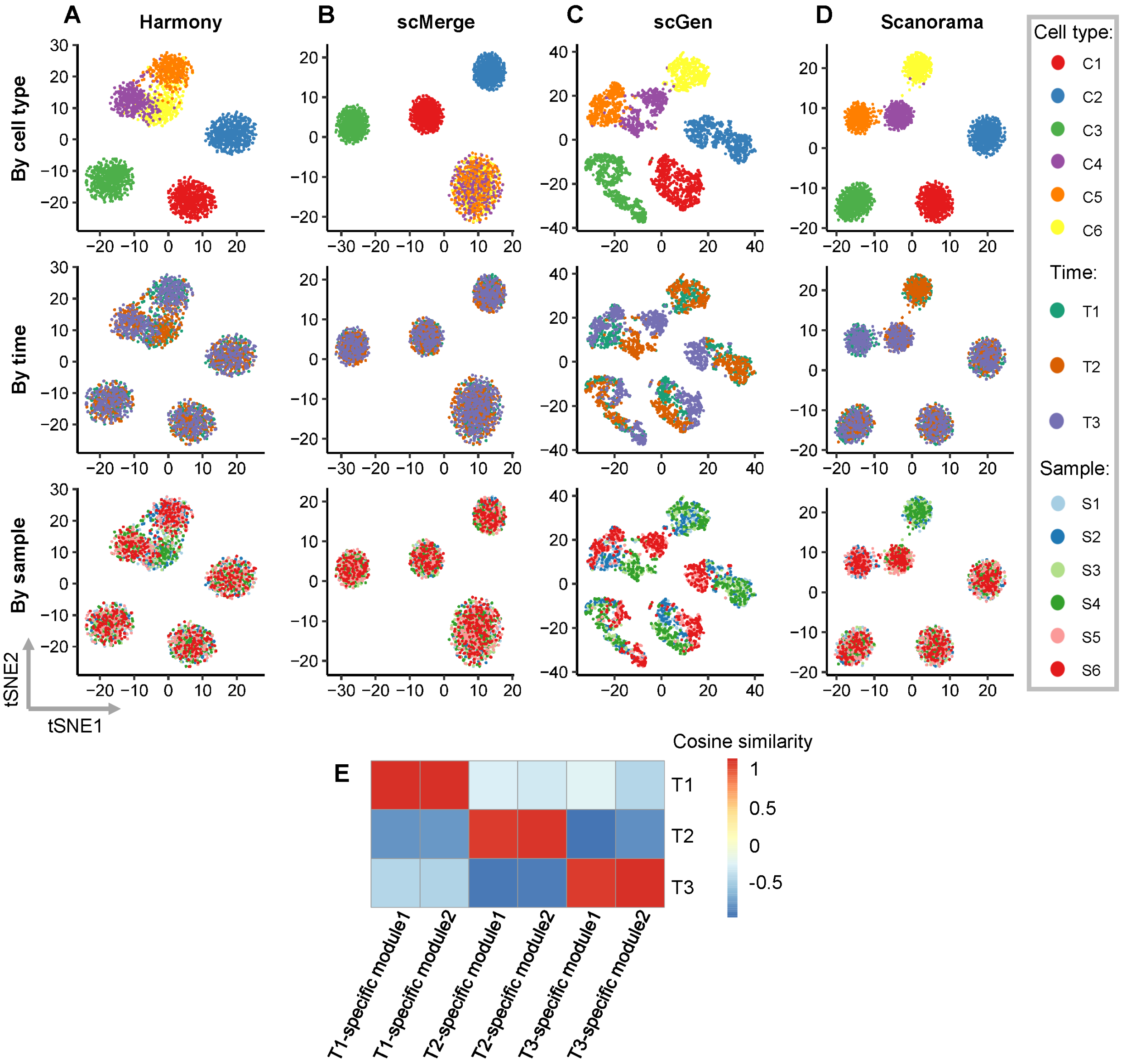


Figure S1: Comparison of integrated data in the simulation study. **A-D**: tSNE plots of simulated cells based on the integrated data by Harmony (**A**), scMerge (**B**), scGen (**C**) and Scanorama (**D**). For each method, three tSNE plots colored by cell type, time point, or sample index are displayed. **E**: Cosine similarity between true time-point effects and membership vectors of condition-specific gene modules obtained by scINSIGHT.

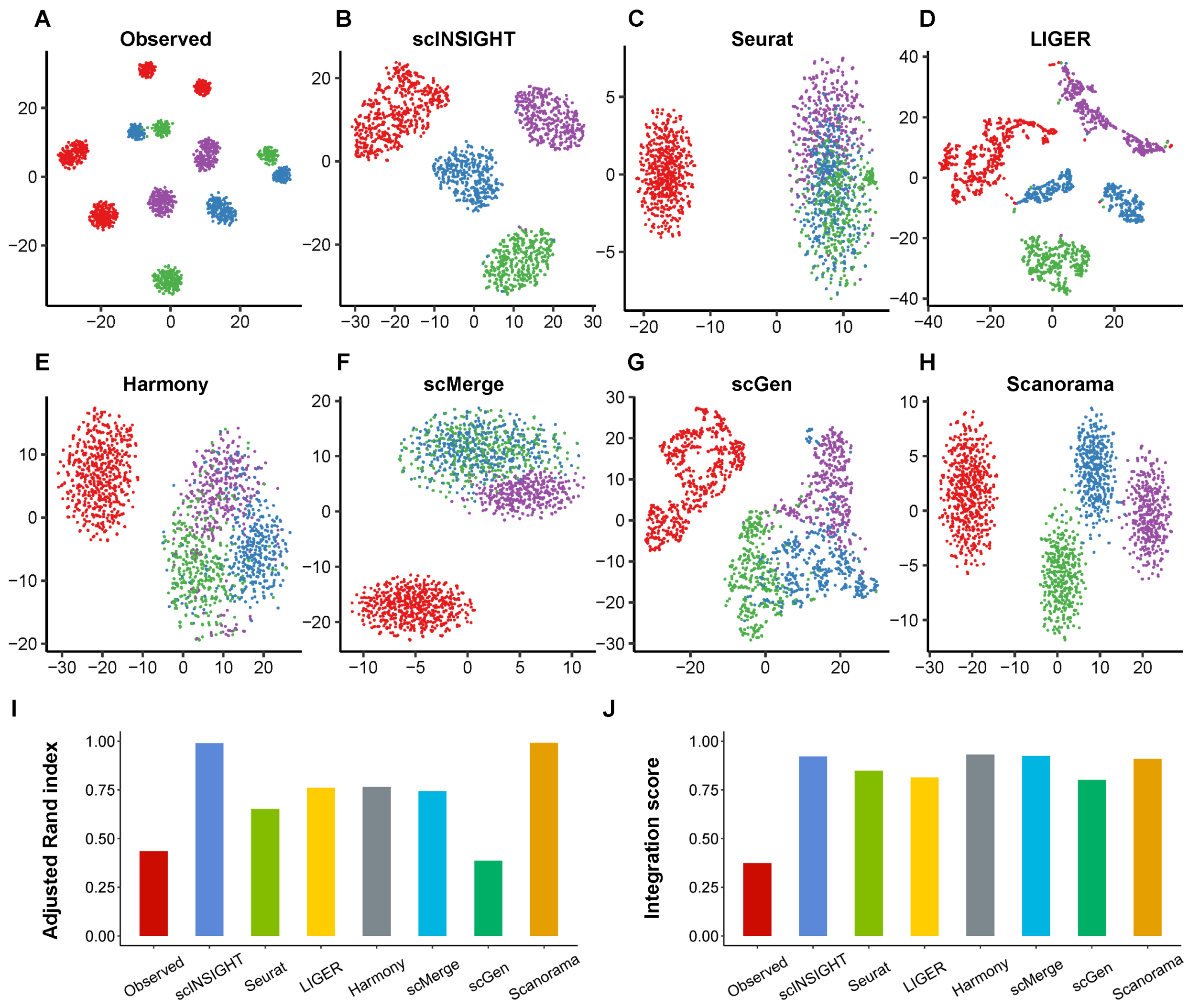


Figure S2: Comparison of observed and integrated data in the simulation study (variant 1). **A-H**: tSNE plots of simulated cells based on the observed (unintegrated) data (**A**) and integrated data by scINSIGHT (**B**), Seurat (**C**), LIGER (**D**), Harmony (**E**), scMerge (**F**), scGen (**G**) and Scanorama (**H**). For each method, tSNE plot colored by cell type is displayed. **I**: Adjusted Rand index calculated using clusters identified from the observed or integrated data. **J**: Integration scores of the observed and integrated data.

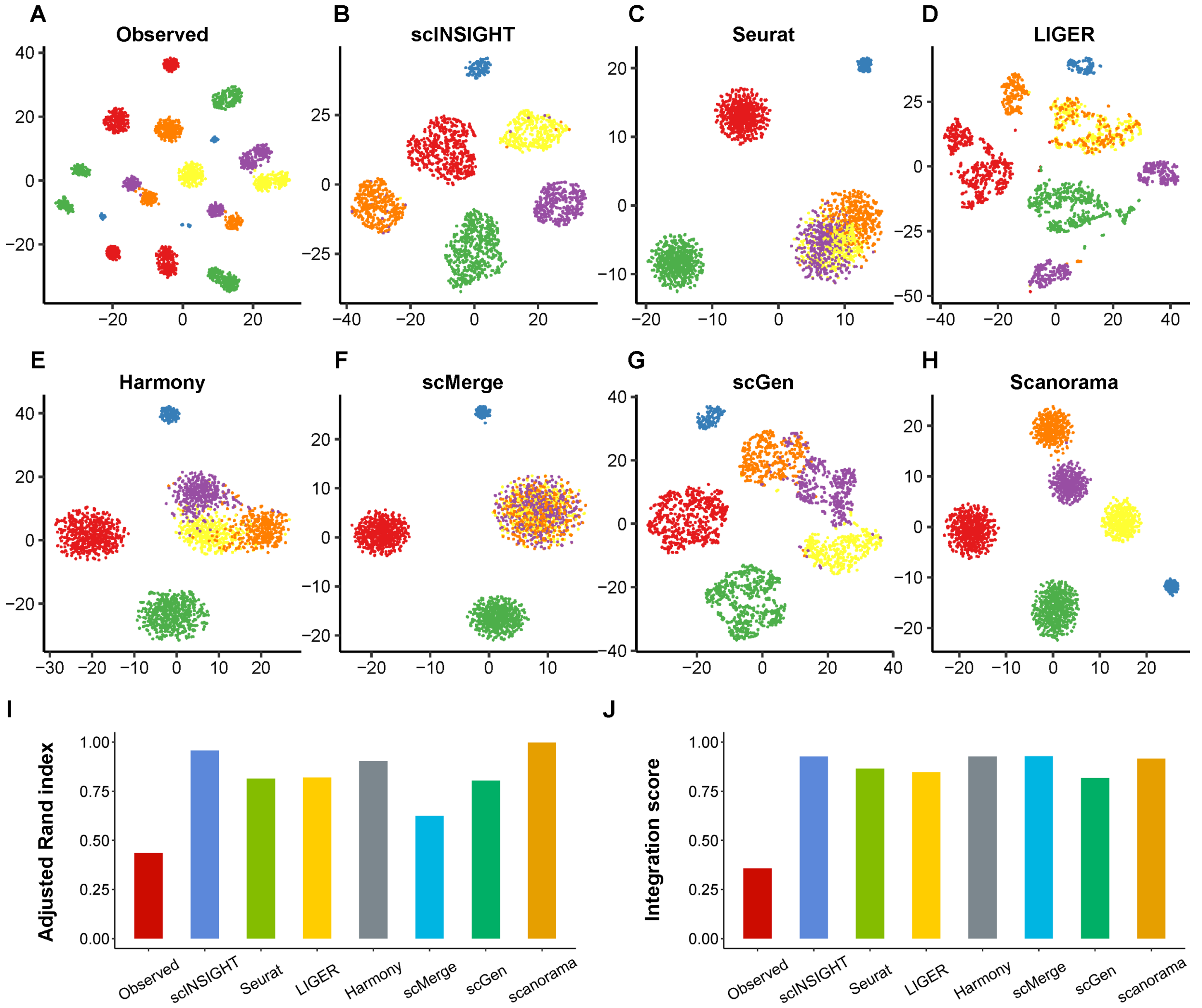


Figure S3: Comparison of observed and integrated data in the simulation study (variant 2). **A-H**: tSNE plots of simulated cells based on the observed (unintegrated) data (**A**) and integrated data by scINSIGHT (**B**), Seurat (**C**), LIGER (**D**), Harmony (**E**), scMerge (**F**), scGen (**G**) and Scanorama (**H**). For each method, tSNE plot colored by cell type is displayed. **I**: Adjusted Rand index calculated using clusters identified from the observed or integrated data. **J**: Integration scores of the observed and integrated data.

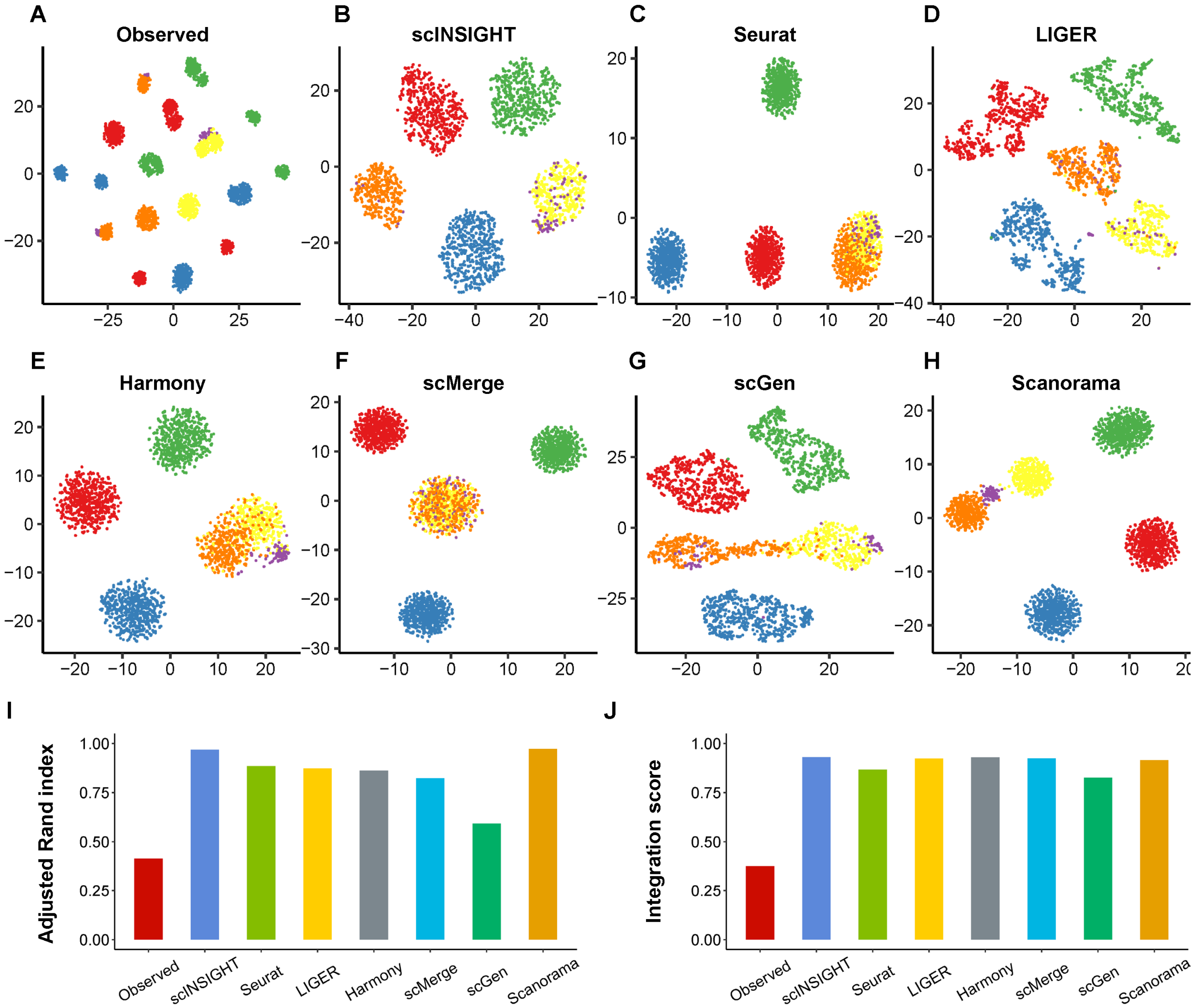


Figure S4: Comparison of observed and integrated data in the simulation study (variant 3). **A-H**: tSNE plots of simulated cells based on the observed (unintegrated) data (**A**) and integrated data by scINSIGHT (**B**), Seurat (**C**), LIGER (**D**), Harmony (**E**), scMerge (**F**), scGen (**G**) and Scanorama (**H**). For each method, tSNE plot colored by cell type is displayed. **I**: Adjusted Rand index calculated using clusters identified from the observed or integrated data. **J**: Integration scores of the observed and integrated data.

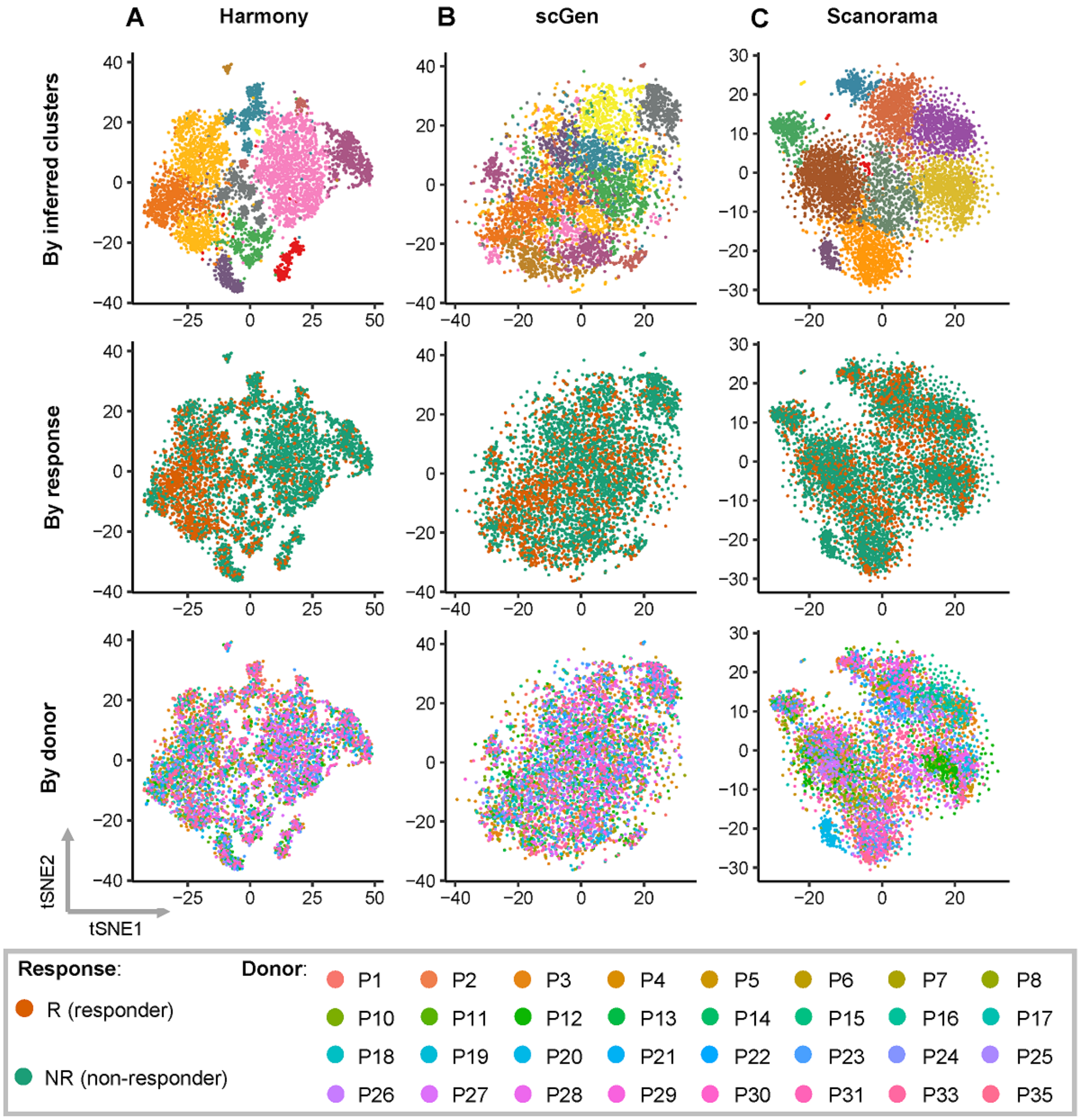


Figure S5: tSNE plots of CD8+ T cells from melanoma patients based on the integrated data by Harmony (**A**), scGen (**B**) and Scanorama (**C**). For each method, three tSNE plots colored by inferred cell cluster, NR/R condition, or donor index are displayed.

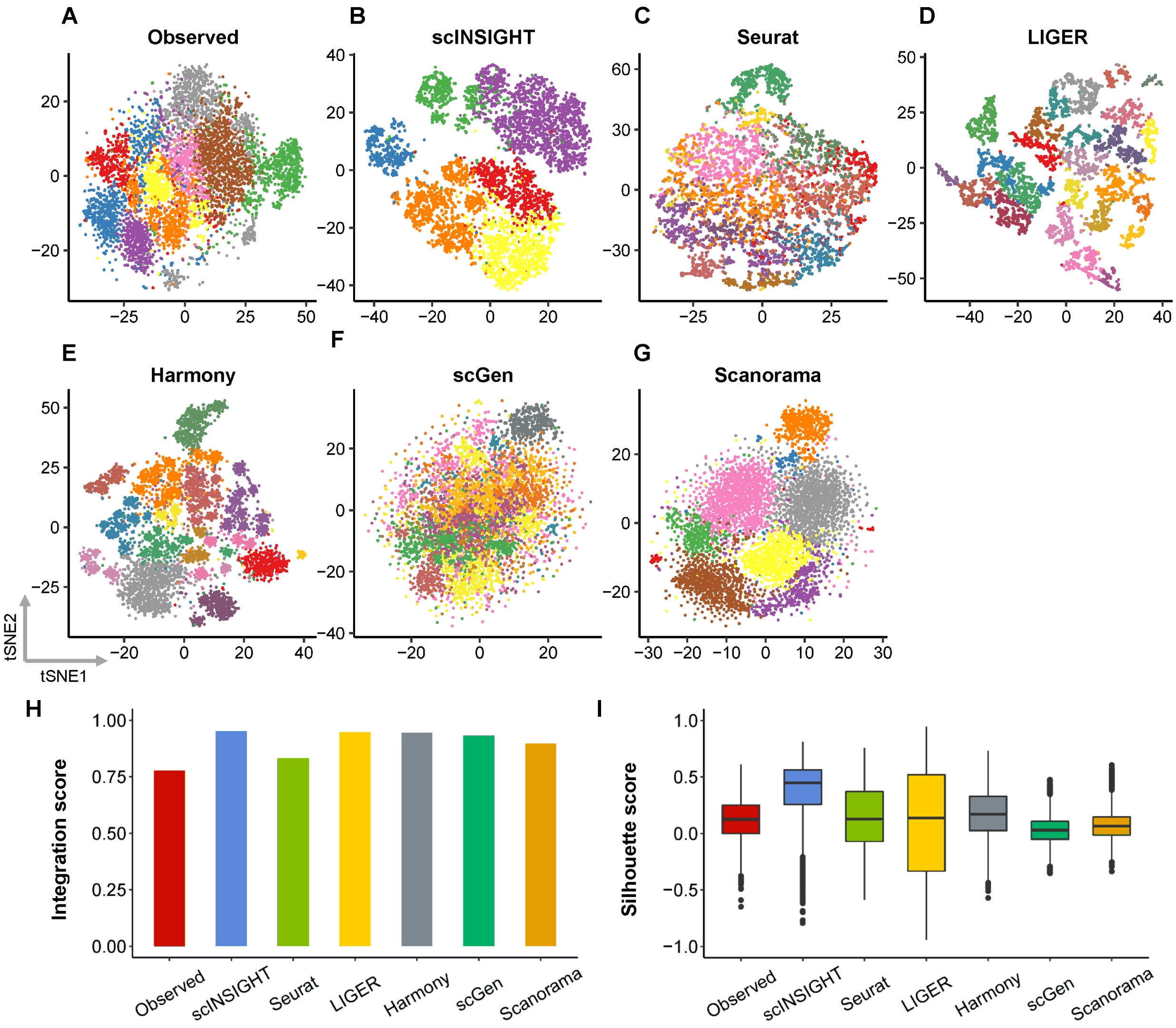


Figure S6: Comparison of observed and integrated data in the melanoma study (using highly variable genes). **A-G**: tSNE plots of simulated cells based on the observed (unintegrated) data (**A**) and integrated data by scINSIGHT (**B**), Seurat (**C**), LIGER (**D**), Harmony (**E**), scGen (**F**) and Scanorama (**G**). Cells are colored by inferred cell cluster. **H**: Adjusted Rand index calculated using clusters identified from the observed or integrated data. **I**: Integration scores of the observed and integrated data.

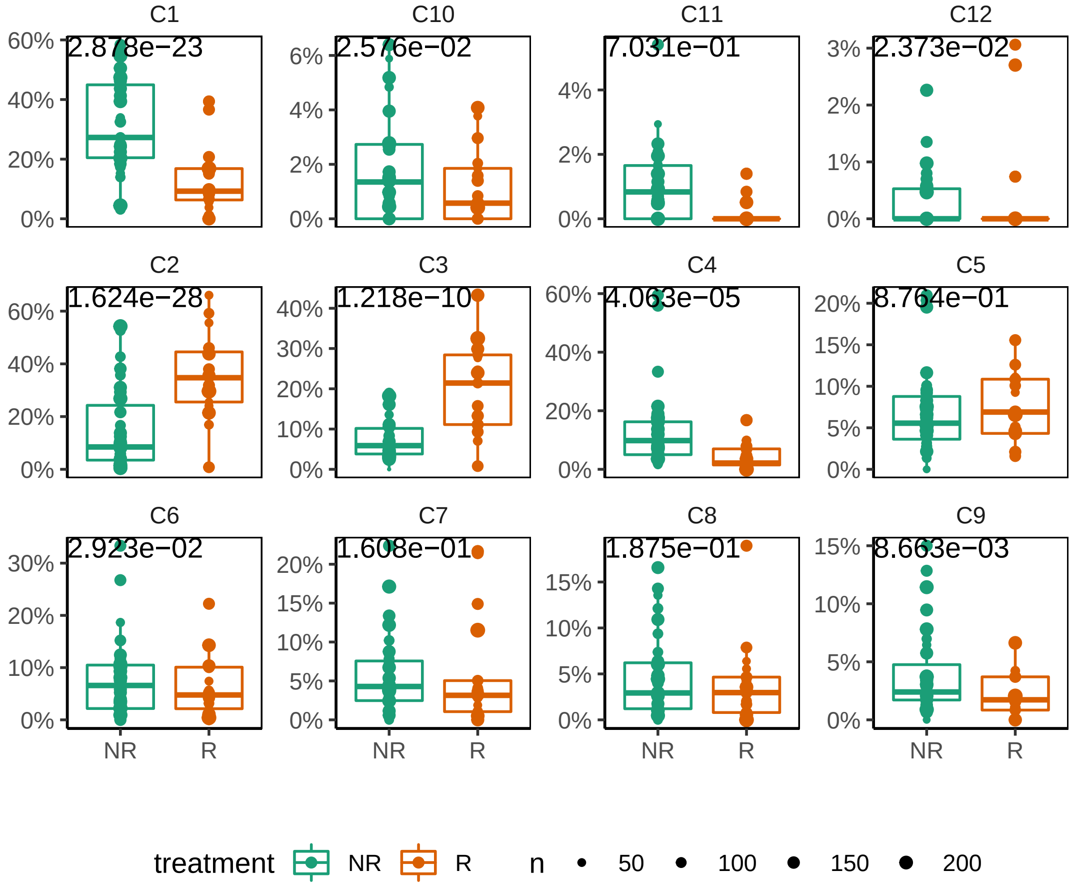


Figure S7: Percentage of the clusters identified by Harmony. P-values indicate significance of association between cluster proportion and response, and were calculated by ANOVA for the logistic regression model.

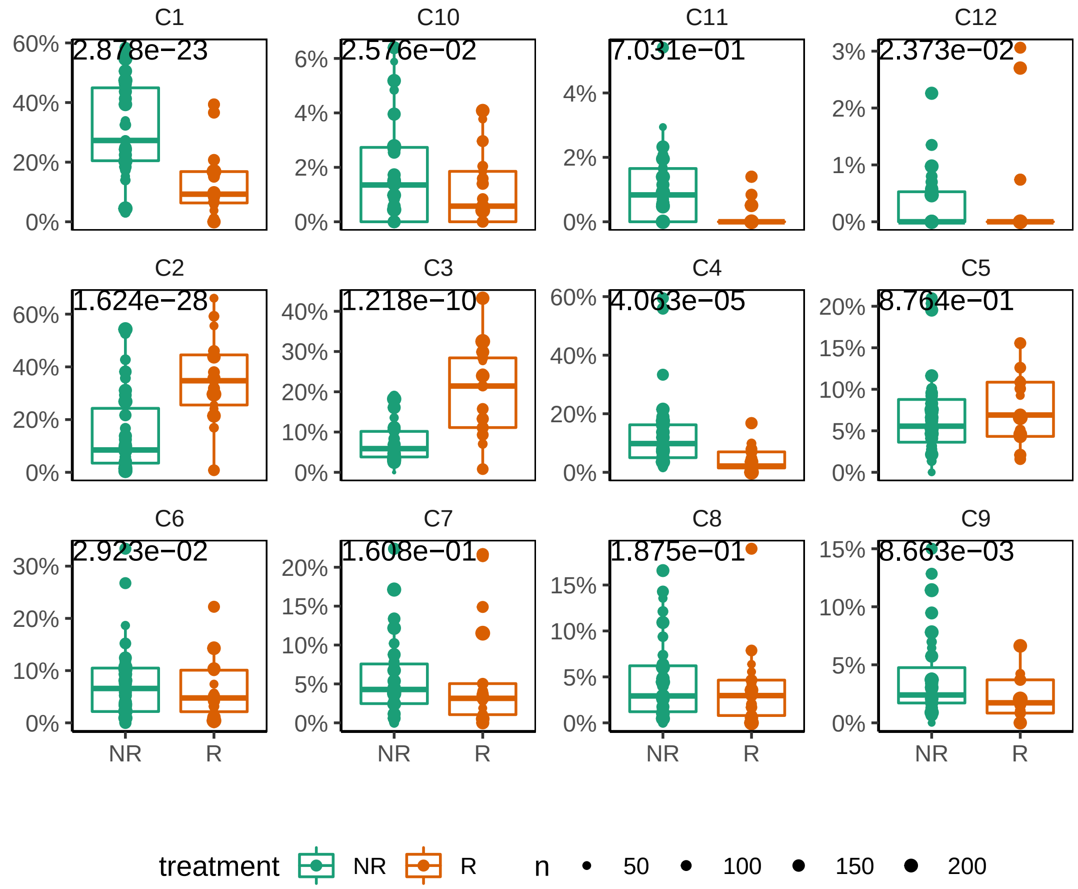


Figure S8: Percentage of the clusters identified by scGen. P-values indicate significance of association between cluster proportion and response, and were calculated by ANOVA for the logistic regression model.

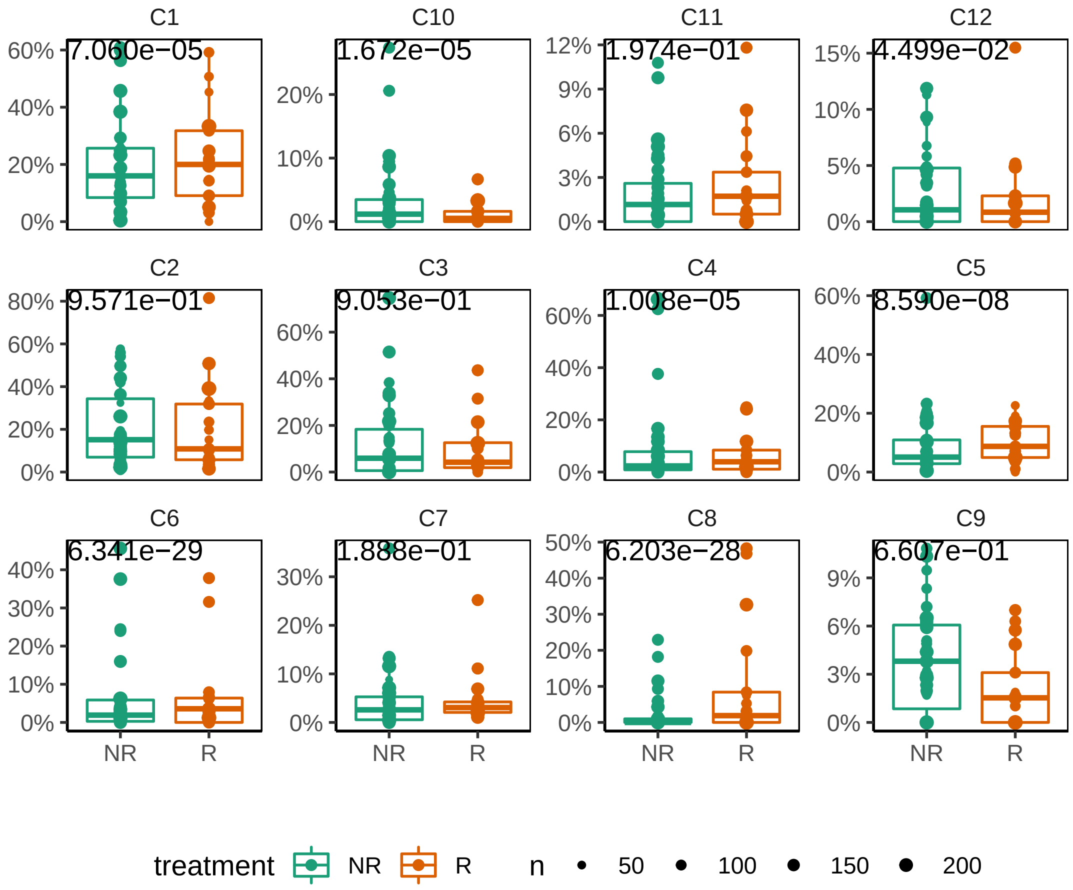


Figure S9: Percentage of the clusters identified by Seurat. P-values indicate significance of association between cluster proportion and response, and were calculated by ANOVA for the logistic regression model.

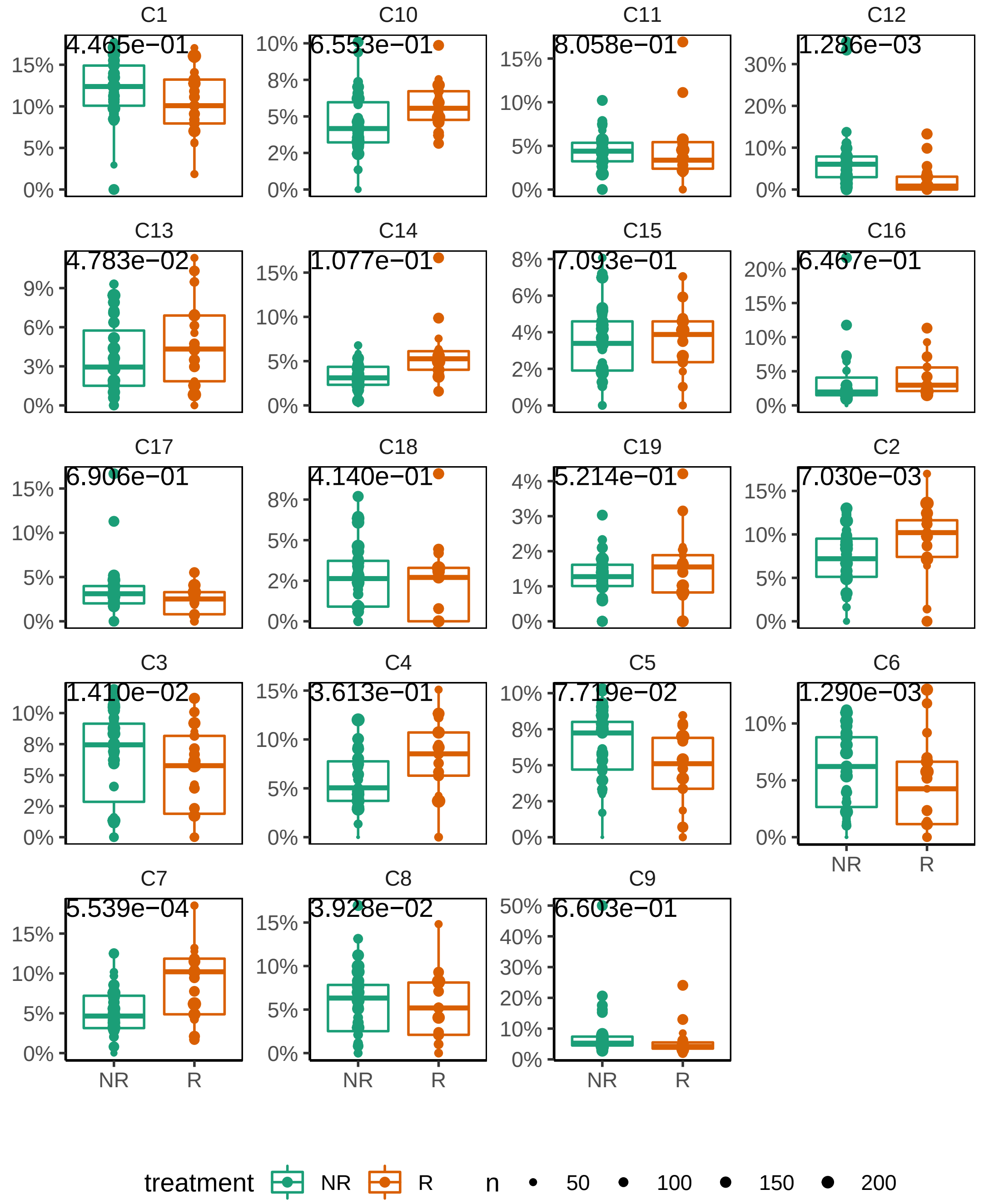


Figure S10: Percentage of the clusters identified by LIGER. P-values indicate significance of association between cluster proportion and response, and were calculated by ANOVA for the logistic regression model.

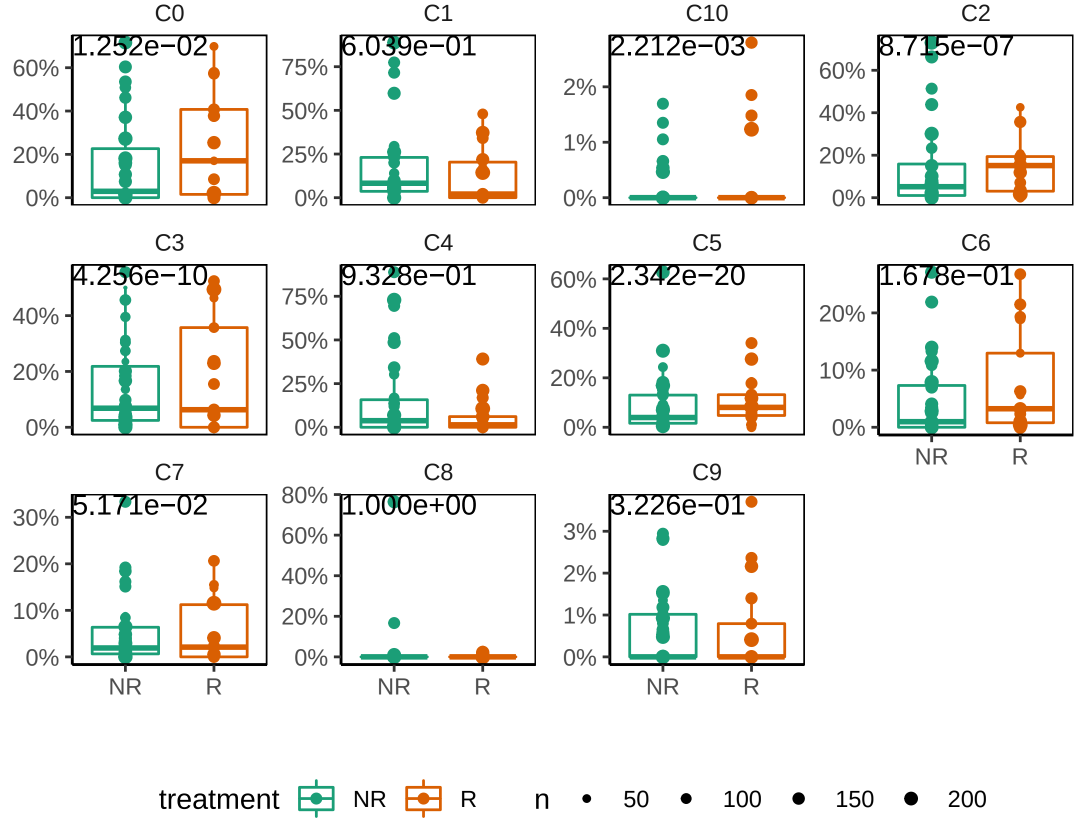
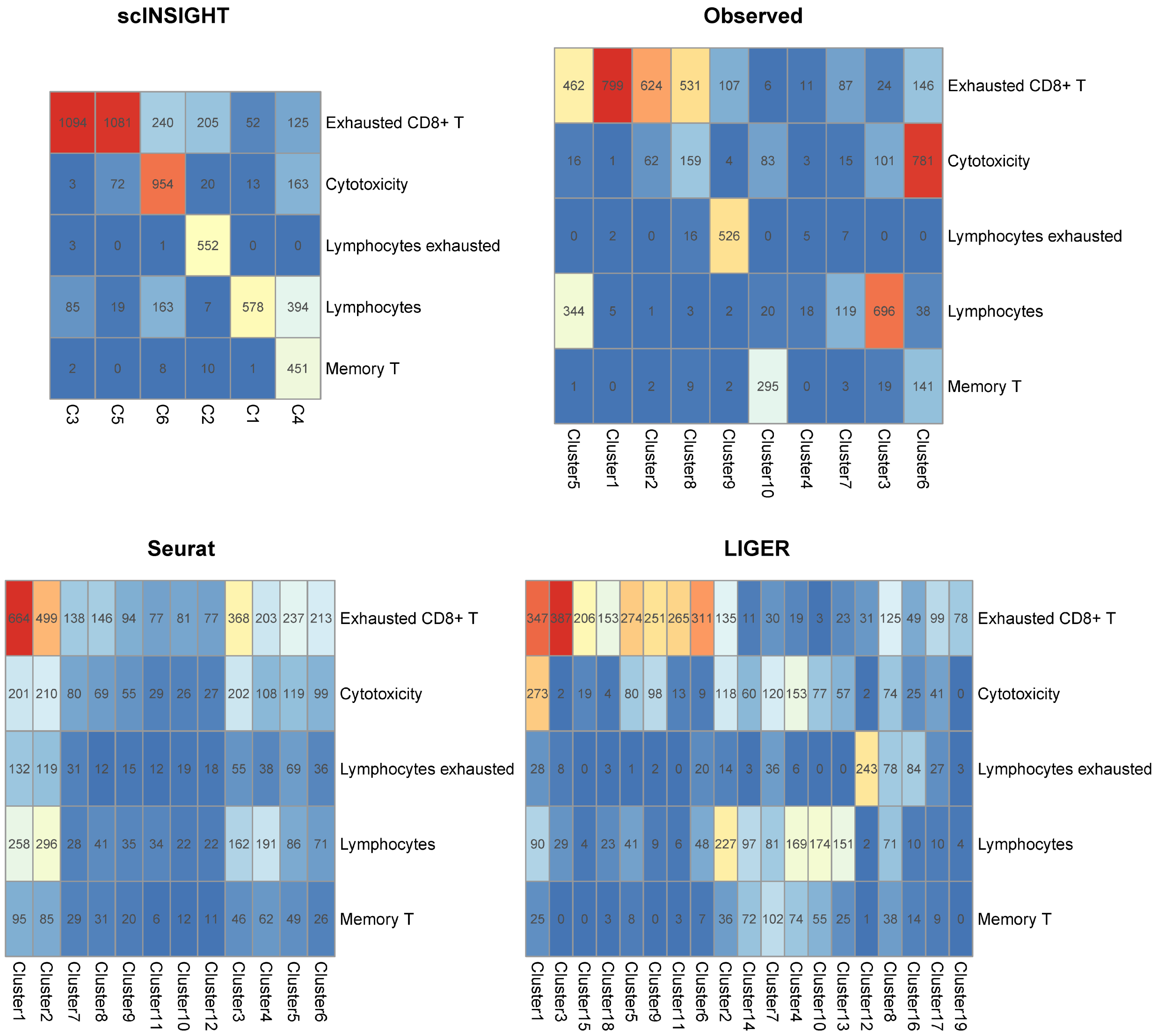


Figure S11: Percentage of the clusters identified by Scanorama. P-values indicate significance of association between cluster proportion and response, and were calculated by ANOVA for the logistic regression model.



scINSIGHT

	C3	C5	C6	C2	C1	C4
Exhausted CD8+ T	1094	1081	240	205	52	125
Cytotoxicity	3	72	954	20	13	163
Lymphocytes exhausted	3	0	1	552	0	0
Lymphocytes	85	19	163	7	578	394
Memory T	2	0	8	10	1	451

Observed

	Cluster5	Cluster1	Cluster2	Cluster8	Cluster9	Cluster10	Cluster4	Cluster7	Cluster3	Cluster6
Exhausted CD8+ T	462	799	624	531	107	6	11	87	24	146
Cytotoxicity	16	1	62	159	4	83	3	15	101	781
Lymphocytes exhausted	0	2	0	16	526	0	5	7	0	0
Lymphocytes	344	5	1	3	2	20	18	119	696	38
Memory T	1	0	2	9	2	295	0	3	19	141

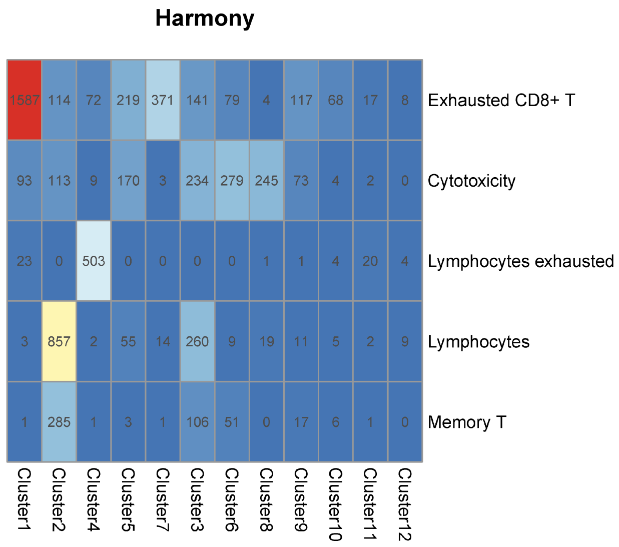
Seurat

	Cluster1	Cluster2	Cluster7	Cluster8	Cluster9	Cluster11	Cluster10	Cluster12	Cluster3	Cluster4	Cluster5	Cluster6
Exhausted CD8+ T	664	499	138	146	94	77	81	77	368	203	237	213
Cytotoxicity	201	210	80	69	55	29	26	27	202	108	119	99
Lymphocytes exhausted	132	119	31	12	15	12	19	18	55	38	69	36
Lymphocytes	258	296	28	41	35	34	22	22	162	191	86	71
Memory T	95	85	29	31	20	6	12	11	46	62	49	26

LIGER

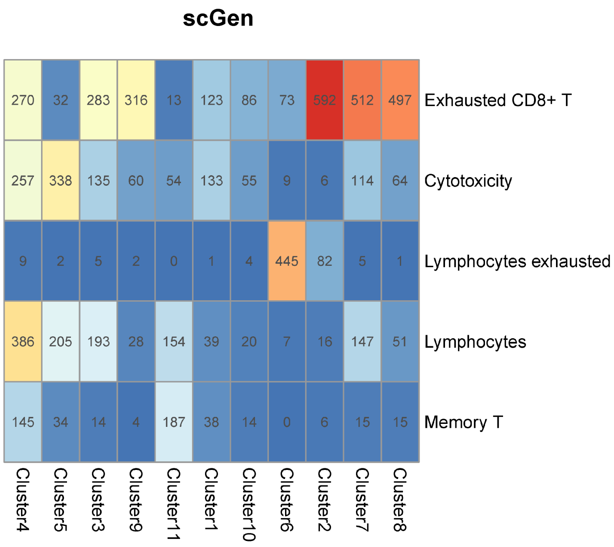
	Cluster1	Cluster3	Cluster15	Cluster18	Cluster5	Cluster9	Cluster11	Cluster6	Cluster2	Cluster14	Cluster7	Cluster4	Cluster10	Cluster13	Cluster12	Cluster8	Cluster16	Cluster17	Cluster19
Exhausted CD8+ T	347	387	206	153	274	251	265	311	135	11	30	19	3	23	31	125	49	99	78
Cytotoxicity	273	2	19	4	80	98	13	9	118	60	120	153	77	57	2	74	25	41	0
Lymphocytes exhausted	28	8	0	3	1	2	0	20	14	3	36	6	0	0	243	78	84	27	3
Lymphocytes	90	29	4	23	41	9	6	48	227	97	81	169	174	151	2	71	10	10	4
Memory T	25	0	0	3	8	0	3	7	36	72	102	74	55	25	1	38	14	9	0

Figure S12: Contingency tables of cell type annotations presented in Sade-Feldman et al (original publication) and computationally inferred cell clusters based on observed data and integrated data by scINSIGHT, Seurat, and LIGER.



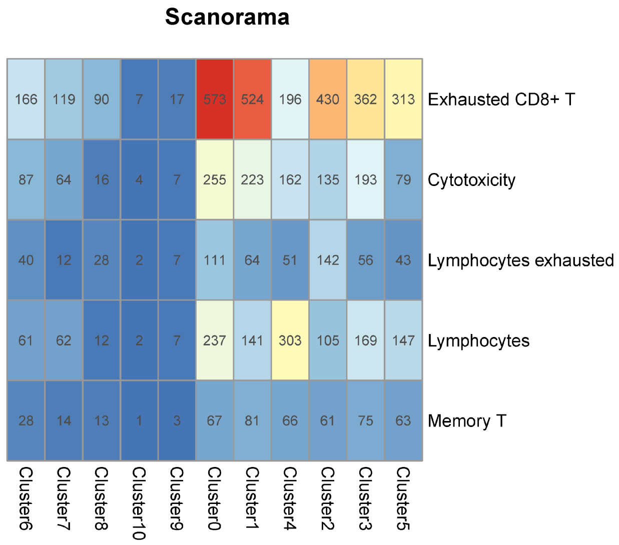
Harmony

	Cluster1	Cluster2	Cluster4	Cluster5	Cluster7	Cluster3	Cluster6	Cluster8	Cluster9	Cluster10	Cluster11	Cluster12
Exhausted CD8+ T	1587	114	72	219	371	141	79	4	117	68	17	8
Cytotoxicity	93	113	9	170	3	234	279	245	73	4	2	0
Lymphocytes exhausted	23	0	503	0	0	0	0	1	1	4	20	4
Lymphocytes	3	857	2	55	14	260	9	19	11	5	2	9
Memory T	1	285	1	3	1	106	51	0	17	6	1	0



scGen

	Cluster4	Cluster5	Cluster3	Cluster9	Cluster11	Cluster1	Cluster10	Cluster6	Cluster2	Cluster7	Cluster8
Exhausted CD8+ T	270	32	283	316	13	123	86	73	592	512	497
Cytotoxicity	257	338	135	60	54	133	55	9	6	114	64
Lymphocytes exhausted	9	2	5	2	0	1	4	445	82	5	1
Lymphocytes	386	205	193	28	154	39	20	7	16	147	51
Memory T	145	34	14	4	187	38	14	0	6	15	15



Scanorama

	Cluster6	Cluster7	Cluster8	Cluster10	Cluster9	Cluster0	Cluster1	Cluster4	Cluster2	Cluster3	Cluster5
Exhausted CD8+ T	166	119	90	7	17	573	524	196	430	362	313
Cytotoxicity	87	64	16	4	7	255	223	162	135	193	79
Lymphocytes exhausted	40	12	28	2	7	111	64	51	142	56	43
Lymphocytes	61	62	12	2	7	237	141	303	105	169	147
Memory T	28	14	13	1	3	67	81	66	61	75	63

Figure S13: Contingency tables of cell type annotations presented in Sade-Feldman et al (original publication) and computationally inferred cell clusters based on observed data and integrated data by Harmony, scGen, and Scanorama.

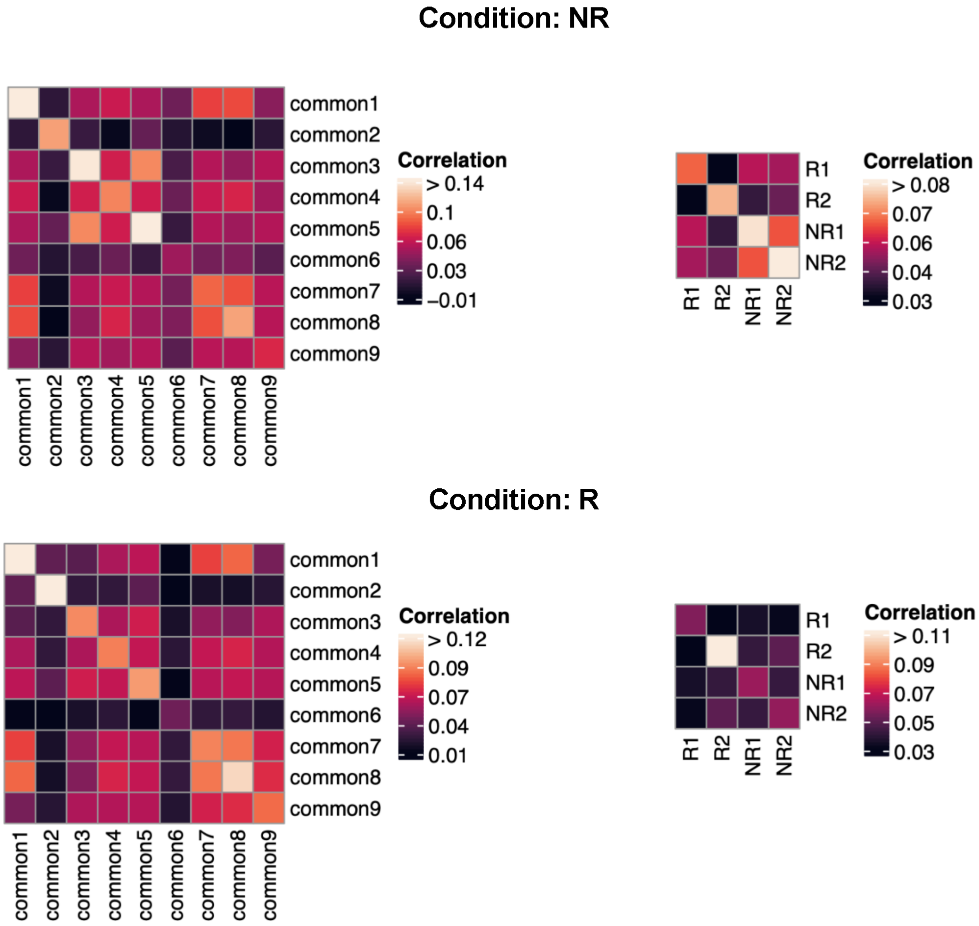
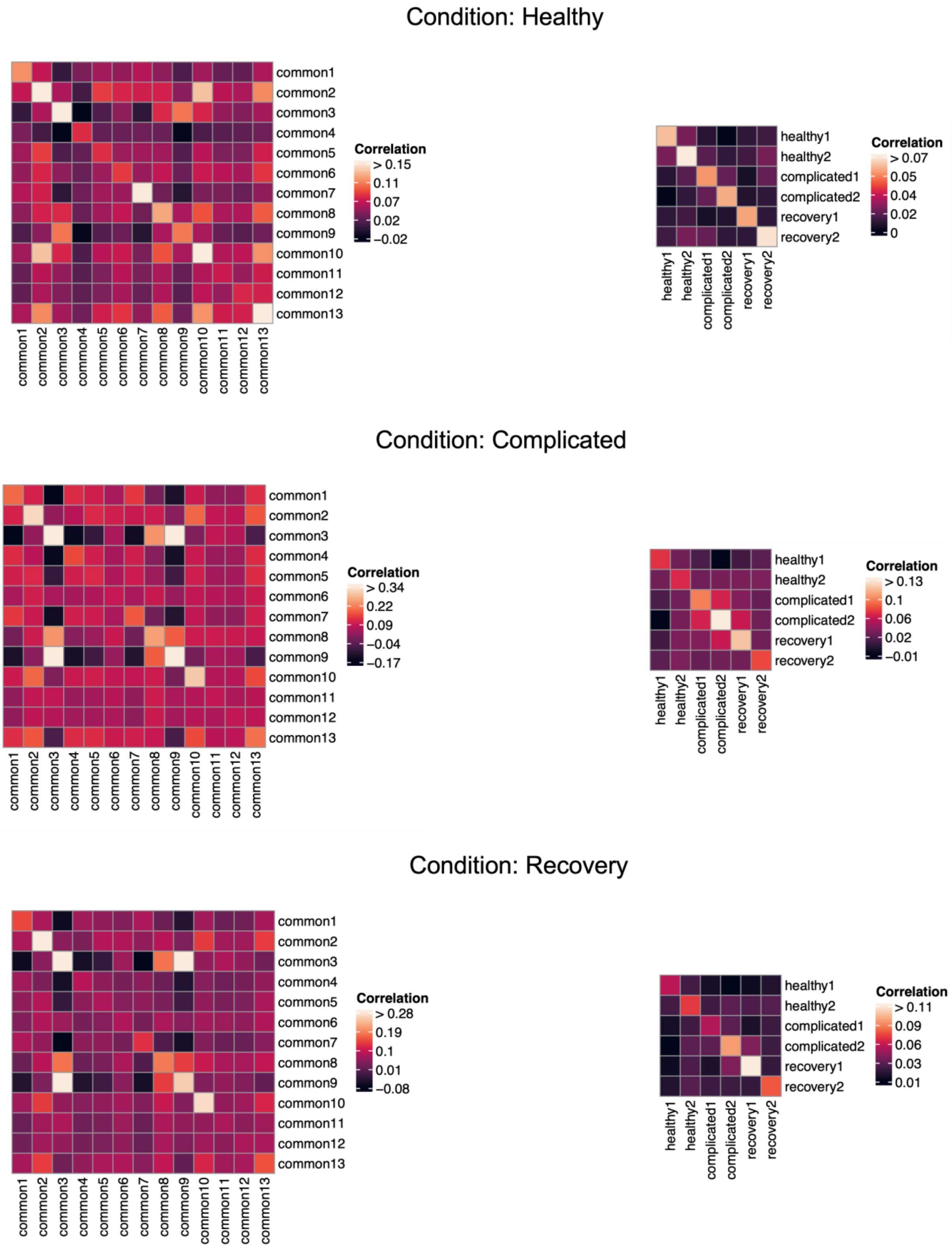


Figure S14: Within-module and between-module co-expression in the melanoma dataset. For each gene module, we selected the top 25 genes with the largest coefficients, and used these genes to calculate within-module and between-module co-expression scores. The within-module co-expression in one condition was calculated as the average pair-wise Spearman's correlation of the top genes in that module, using all cells from that condition. The between-module co-expression in one condition was calculated as the average Spearman's correlation between top genes in the two modules, using all cells from that condition.


Condition: Healthy

Condition: Complicated

Condition: Recovery

Figure S15: Within-module and between-module co-expression in the COVID-19 dataset.

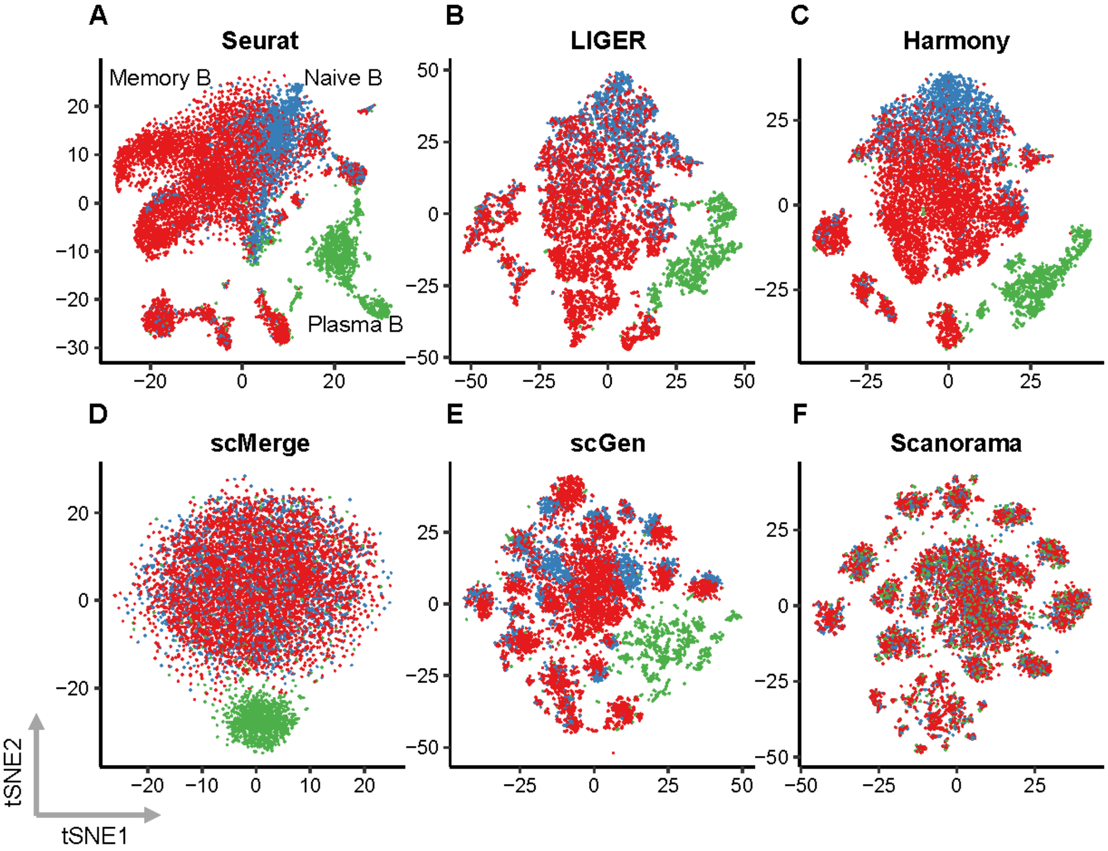


Figure S16: tSNE plots of B cells based on integrated data by Seurat (**A**), LIGER (**B**), Harmony (**C**), scMerge (**D**), scGen (**E**) and Scanorama (**F**). Cells are colored by classified cell type (by SingleR).

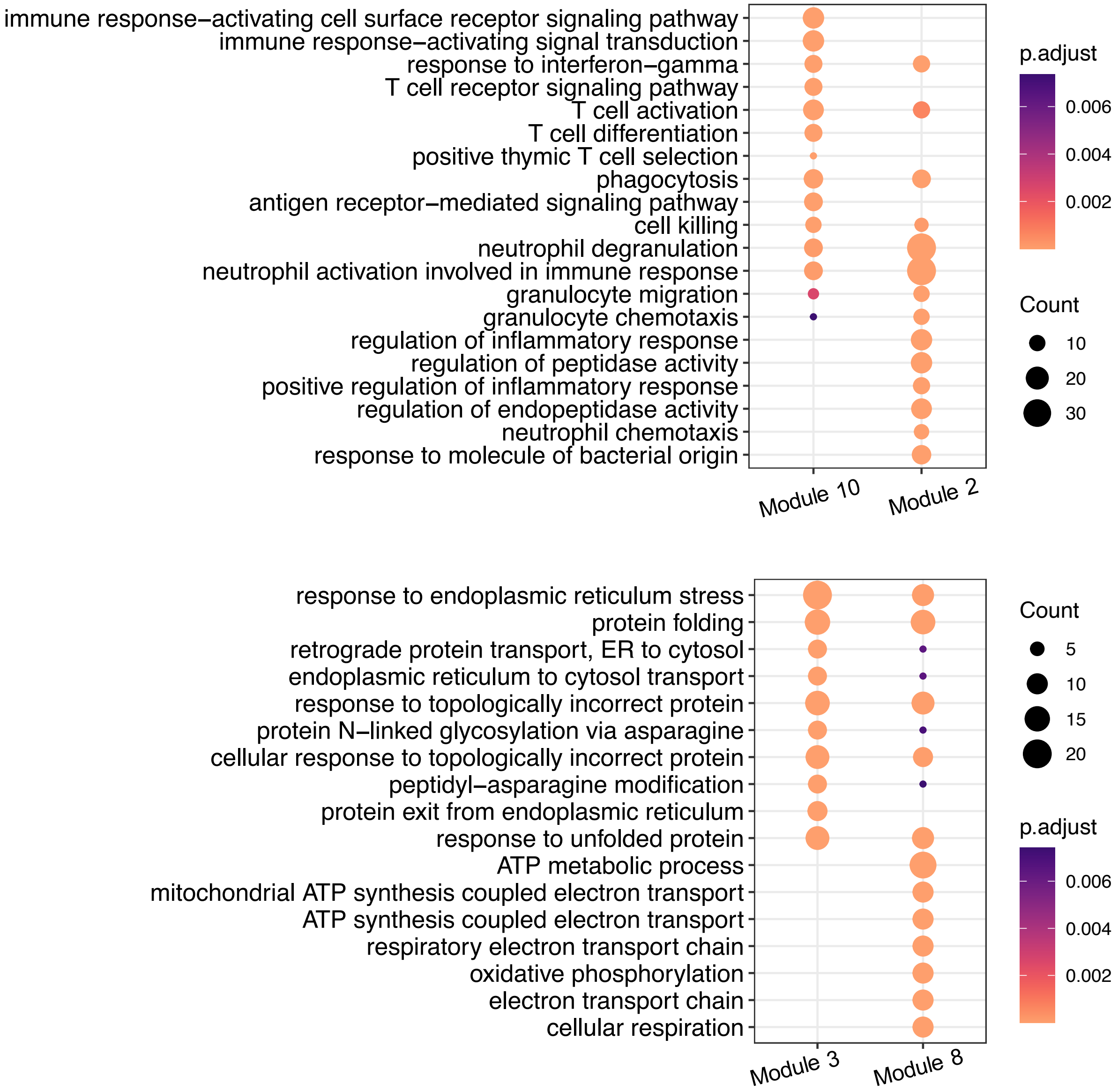
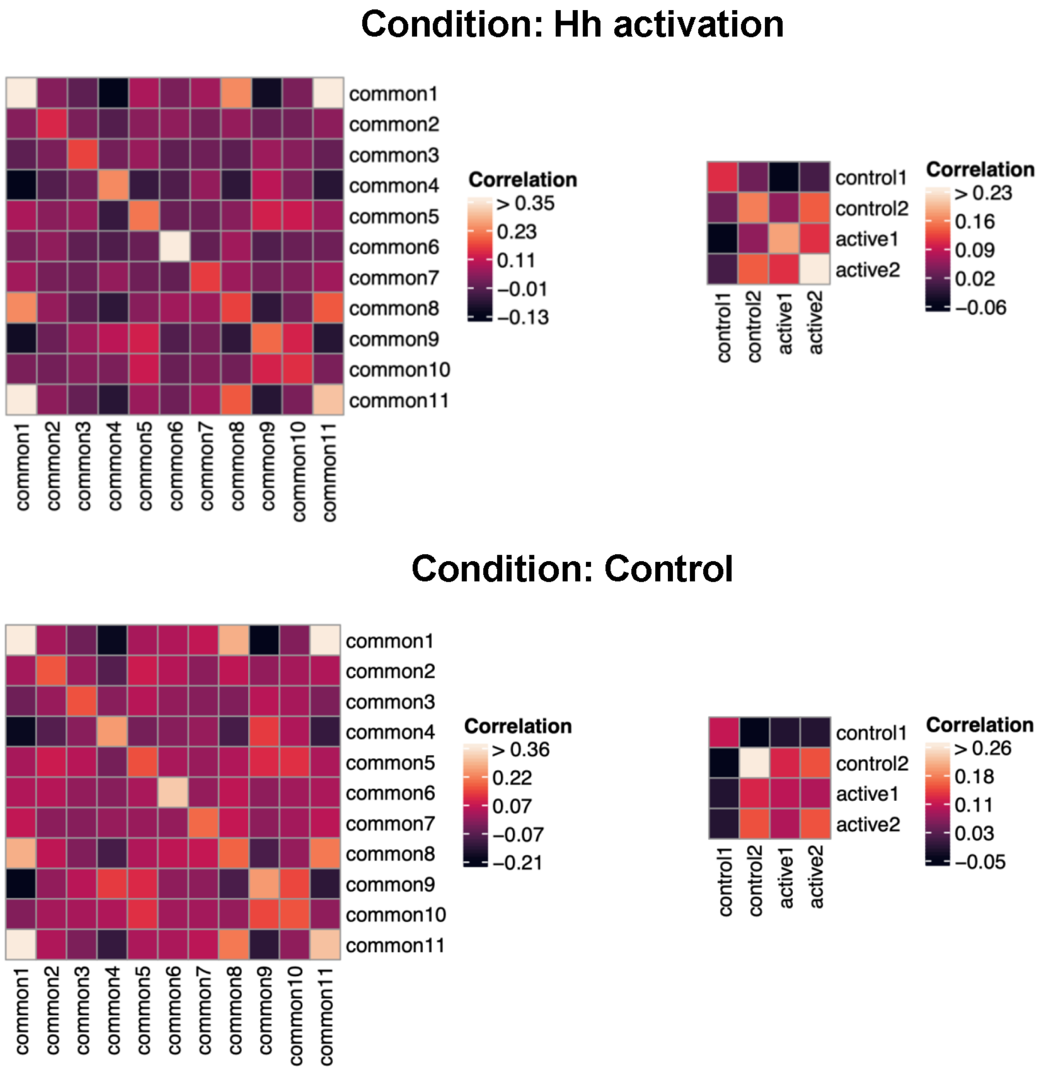


Figure S17: Top enriched GO terms in common gene modules (10, 2, 3, and 8) identified by scINSIGHT.



Condition: Hh activation

Condition: Control

Figure S18: Within-module and between-module co-expression in the Wound healing dataset.

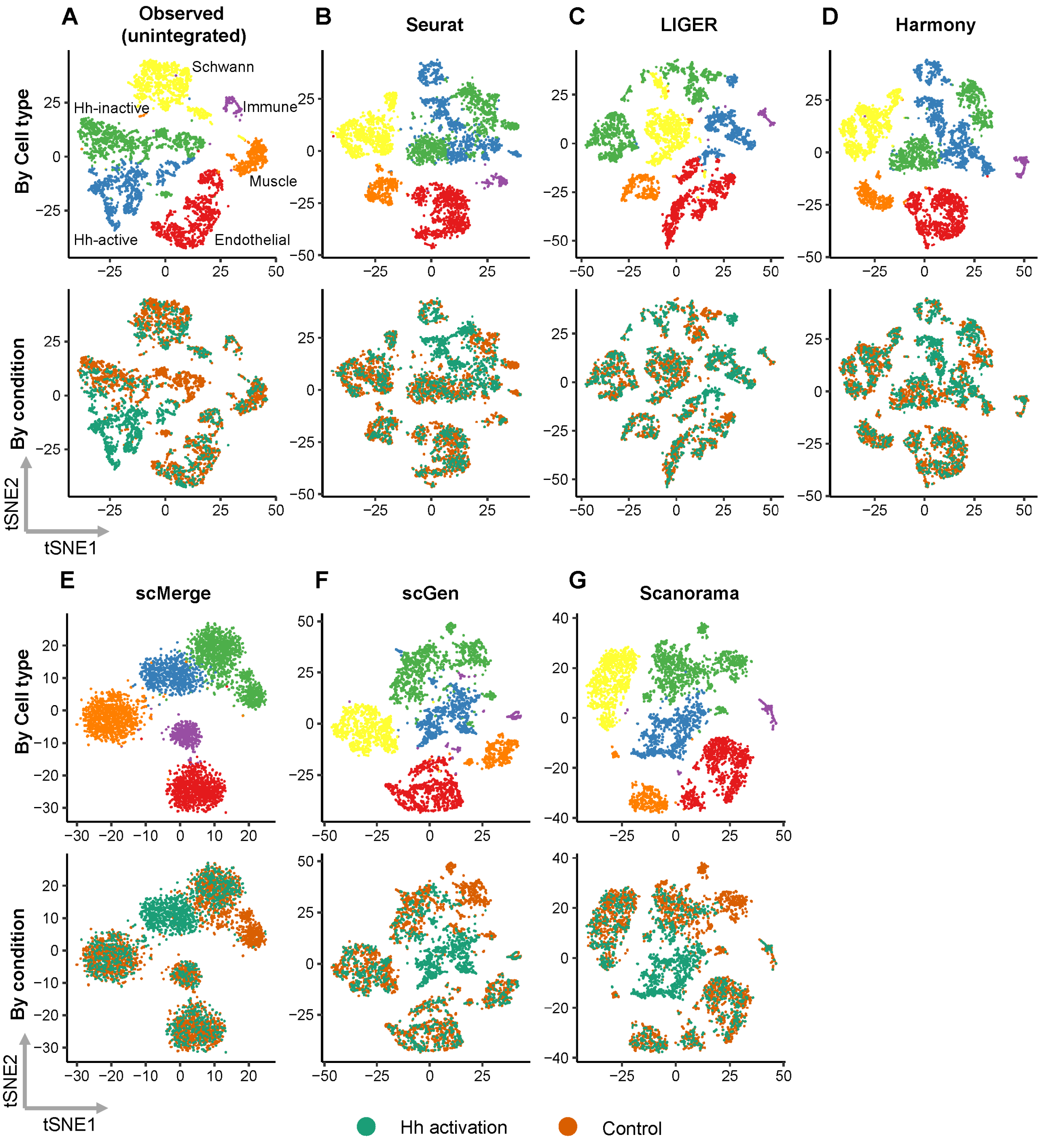


Figure S19: tSNE plots of dermal cells based on observed data (**A**) and integrated data by Seurat (**B**), LIGER (**C**), Harmony (**D**), scMerge (**E**), scGen (**F**), and Scanorama (**G**).

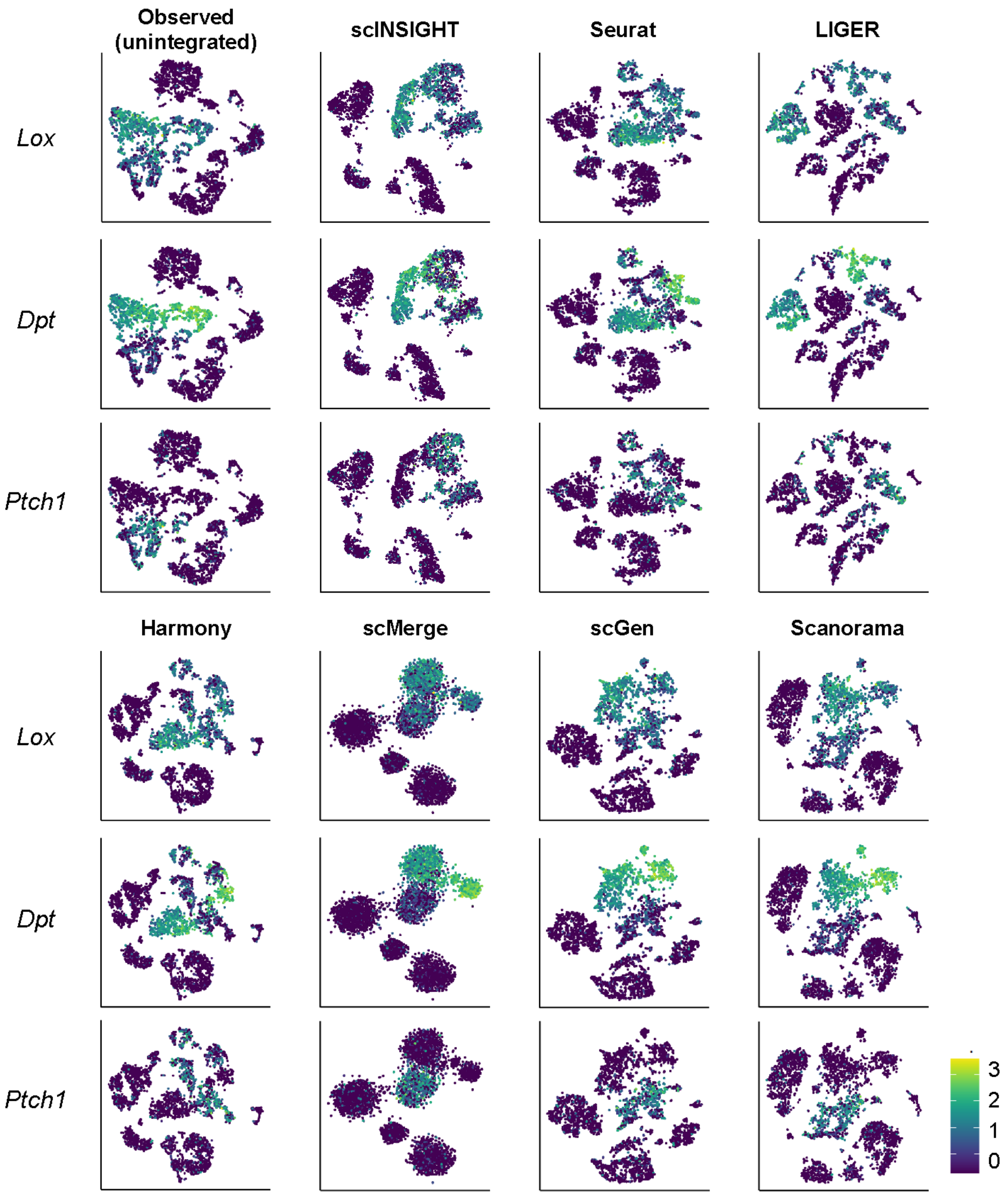


Figure S20: tSNE plots of observed and integrated data colored by scaled expression of fibroblast signatures (*Lox*, *Dpt*, and *Ptch1*).

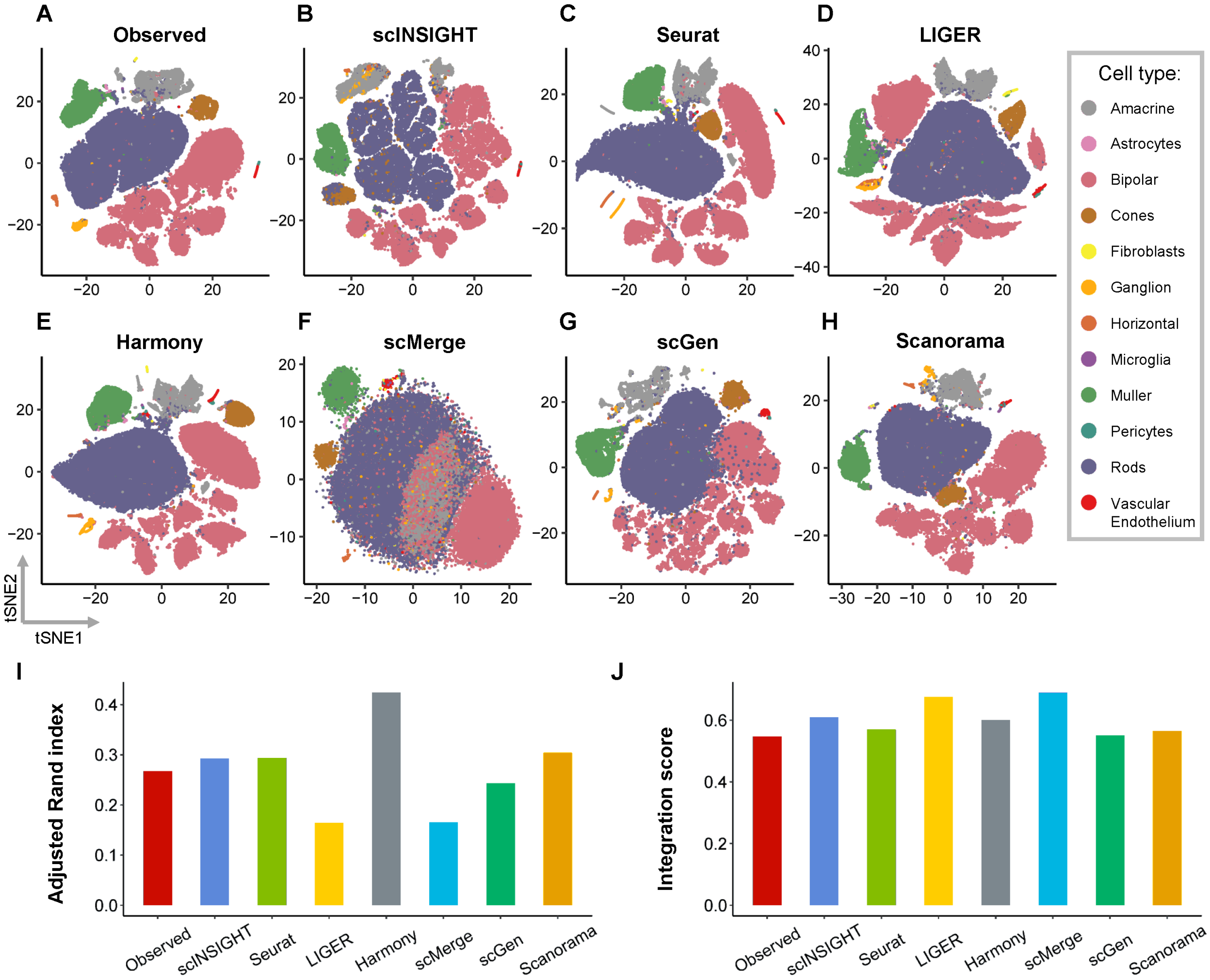


Figure S21: Comparison of observed and integrated data in the mouse retina study. **A-H**: tSNE plots of single cells based on the observed (unintegrated) data (**A**) and integrated data by scINSIGHT (**B**), Seurat (**C**), LIGER (**D**), Harmony (**E**), scMerge (**F**), scGen (**G**), and Scanorama (**H**). Cells are colored by cell type. **I**: Adjusted Rand index calculated using clusters identified from the observed or integrated data. **J**: Integration scores of the observed and integrated data.

Table S1: Running time and memory usage of scINSIGHT and the other six integration methods. For scINSIGHT, the recorded time on the top was time used to run scINSIGHT with different values of K, and the recorded time on the bottom (in the parentheses) was time used to select regularization parameters.

Dataset		scINSIGHT	Seurat	LIGER	Harmony	scMerge	scGen	Scanorama
Simulation 3000 cells 6 samples 3 conditions	Memory	5.91G	7.96G	1.49G	1.10G	2.37G	12.06G	6.72G
	Time	12479.10s (3751.98s)	148.37s	524.57s	3.35s	528.60s	87.59s	4.43s
Melanoma 6350 cells 48 samples 2 conditions	Memory	17.70G	10.45G	9.75G	11.85G	▨	23.06G	14.45G
	Time	6945.26s (3308.20s)	105.68s	340.34s	41.67s	▨	296.70s	8.01s
COVID-19 9741 cells 13 samples 3 conditions	Memory	14.52G	13.25G	4.00G	5.16G	5.88G	18.76G	11.65G
	Time	6753.42s (3733.60s)	702.68s	697.63s	32.47s	209.16s	494.31s	9.55s
Wound healing 4680 cells 2 samples 2 conditions	Memory	7.63G	3.15G	2.26G	3.50G	3.61G	16.16G	9.75G
	Time	2385.18s (849.12s)	60.65s	458.36s	16.75s	78.32s	207.95s	1.92s
Mouse retina 71638 cells 2 samples 2 conditions	Memory	49.5G	23.75G	15.55G	16.25G	54.65G	46.36G	29.55G
	Time	80221.78s (50878.43s)	6381.78s	14910.96s	140.58s	1532.17s	2457.38s	55.82s